Insulin Binding and Removal by Livers of Genetically Obese Rats

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SUMMARY

Livers from 9–10-wk-old genetically obese and hyperinsulinemic (*fa/fa*) rats contained more DNA, proteins, and lipids than livers of age-matched controls. Actually, the increase in liver mass of *fa/fa* rats was mainly due to an increased cell number. On perfusion with insulin (1.75–16 nM), livers of obese *fa/fa* rats removed 35–40% less insulin per gram tissue than control livers. When calculated on a per organ basis, removal capacity was, however, similar in livers of control and obese *fa/fa* rats. The binding of ^{125}I-insulin to as well as the insulin removal by isolated hepatocytes was also assessed. Contrary to previous unexpected data in which the downregulation of insulin receptors by hyperinsulinemia was reported not to prevail in hepatocytes from obese *fa/fa* rats, it was found that the binding capacity of hepatocytes from *fa/fa* rats was decreased by 45% without alteration of the binding affinity. Moreover, in hepatocytes from obese *fa/fa* rats, both binding and removal of insulin were lowered to an analogous extent. It is concluded that livers of obese *fa/fa* rats behave, with regard to insulin binding and removal, as those of other hyperinsulinemic obese animals. DIABETES 32:605–609, July 1983.

In the many obese hyperinsulinemic animals studied, insulin binding to target tissues is decreased, at least at the late phase of the syndrome (for review, see ref. 1). In this respect, the liver of genetically obese *fa/fa* rat constitutes an apparent exception. Indeed, in this species, increased plasma insulin levels were reported not to result in significant downregulation of the insulin receptor sites.[2,3] On the other hand, previous studies have shown that a reduced efficiency in the hepatic removal of insulin was also a feature of some hyperinsulinemic states such as those found in obese hyperglycemic (*ob/ob*) mice and spontaneously obese rats.[4,5] In obese hyperglycemic mice in particular, in vitro hepatic insulin removal was found to be all the smaller that hyperinsulinemia measured in vivo prior to the experiments was greater. This abnormality was corrected when hyperinsulinemia was reduced toward or to normal.[4] Moreover, it has been shown in isolated hepatocytes from normal rats that receptor-mediated insulin degradation accounts for virtually all insulin degradation in vitro and that the rate of degradation was proportional to the insulin receptor occupancy.[6] The aim of the present study was therefore to measure insulin removal and binding in livers of lean and obese *fa/fa* rats to determine whether changes in insulin removal could possibly occur in the absence of insulin receptor site alterations as could be thought when referring to presently available data.

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MATERIALS AND METHODS

Animals. Nine-to-ten-week-old genetically obese female Zucker (*fa/fa*) rats and their lean littermate controls were purchased from the Centre de Sélection et d'Elevage d'Animaux de Laboratoire (CNRS, Orléans, France). All animals were fed ad libitum with standard laboratory chow and placed in animal quarters with constant temperature (24°C) and fixed 12-h light cycle.

Insulin removal by perfused livers. The livers were perfused in situ[7] for 40 min with recirculating Krebs-Ringer bicarbonate buffer containing 3% bovine serum albumin, 20% washed bovine erythrocytes, and varying concentrations of porcine insulin. Aliquots of the perfusion medium were taken at several time intervals for the determination of immunoreactive insulin.

Insulin binding to isolated hepatocytes. Liver cells were isolated as previously described[8] and preincubated for 30 min at 30°C in a Krebs-Ringer bicarbonate buffer containing 3% bovine serum albumin and 150 mg/dl glucose. Cells were then centrifuged, resuspended (10^6 cells/ml) and incubated at 30°C in triplicates in the same medium supplemented with 10 mg/dl bacitracin, 0.025–0.03 nM ^{125}I-insulin, and various

TABLE 1
Basic characteristics of livers from lean and genetically obese *fa/fa* rats

Measurements	Lean	*fa/fa*
Body wt (g)	192 ± 6	341 ± 7*
Liver wt (g)	7.7 ± 0.3	11.0 ± 0.3*
Liver wt/body wt (%)	4.02 ± 0.07	3.22 ± 0.04*
Liver dry wt/liver wet wt (%)	31.3 ± 0.1	31.9 ± 0.6
DNA		
(mg/g)	2.75 ± 0.08	2.61 ± 0.07
(mg/liver)	21.1 ± 0.8	28.7 ± 0.9*
Proteins		
(mg/g)	186 ± 4	196 ± 3
(mg/liver)	1385 ± 57	2154 ± 62*
Lipids		
(mg/g)	48 ± 1	68 ± 2*
(mg/liver)	370 ± 18	742 ± 31*

Nine-to-ten-week-old lean and *fa/fa* rats were anesthetized with pentothal sodium (80 mg/kg). The livers were rapidly excised and weighed. Pieces of tissue were frozen into liquid nitrogen for subsequent analysis as described under MATERIALS AND METHODS. Values are means ± SE of 10 rats.
*$P < 0.0005$.

concentrations of unlabeled porcine insulin. Immediately after sampling, the cells were separated from the incubation medium by centrifugation through oil (dionylphtalate-dibutylphtalate 1:3). Aliquots of cell suspension and cell pellets were counted in a gamma scintillation spectrophotometer (Packard Instrument, Inc., Illinois). The cell-associated radioactivity determined for cells incubated in the presence of 1 μM native insulin was considered to represent the nonspecific binding and subtracted from total binding to obtain specific binding values. The cell viability (assessed by trypan blue exclusion) of all the cell preparations used for this study exceeded 94% at the end of the experiment.

Measurements. Pieces of frozen liver were used for the determination of DNA,[9] protein,[10] and lipid[11] content. Immunoreactive insulin was measured according to Herbert et al.[12] against standard curves of porcine insulin diluted in Krebs-Ringer bicarbonate buffer containing 3% bovine serum albumin.

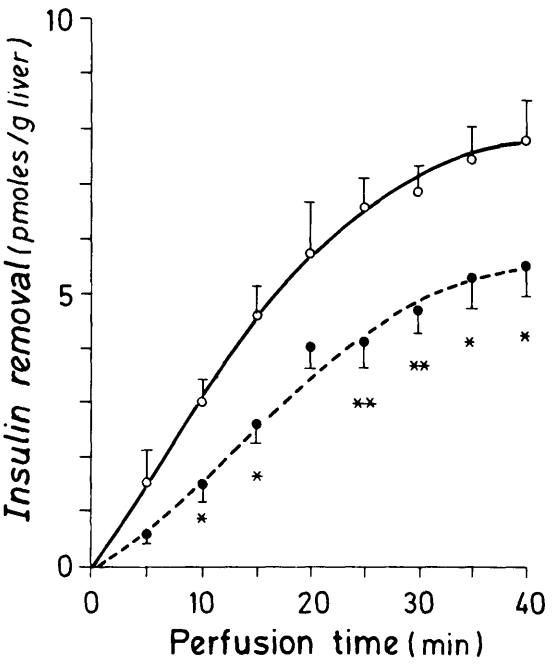

FIGURE 1. Time course of insulin removal by perfused livers of lean (full line) and obese *fa/fa* rats (dotted line). Liver perfusions were performed as described in MATERIALS AND METHODS. The initial concentration of porcine insulin added to the medium was 1.75 nM. Values are means ± SE of six animals. *$P < 0.05$; **$P < 0.025$.

Chemicals. Porcine insulin was purchased from Novo Industri A/S (Bagsvaerd, Denmark), porcine ^{125}I-insulin (100 μCi/μg) from CIS Radium Chemie AG (Teufen, Switzerland) and anti-porcine insulin guinea pig serum (0.74 mU/μl) from

TABLE 2
Insulin removal by perfused livers of lean and obese *fa/fa* rats

Animals	Perfusion time (min)	Initial concentration of insulin 1.75 mM	3.5 mM	8 mM	16 mM
		Insulin removed (pmol/g liver)			
Lean	15	4.6 ± 0.7	7.5 ± 0.4	17.3 ± 2.6	20.1 ± 1.2
fa/fa	15	2.6 ± 0.3*	5.7 ± 0.3†	10.4 ± 2.2‡	16.4 ± 1.9
Lean	25	6.6 ± 0.6	11.1 ± 0.7	28.2 ± 2.9	36.0 ± 3.8
fa/fa	25	4.1 ± 0.5†	8.2 ± 0.5*	19.8 ± 2.6‡	22.7 ± 3.1*
Lean	35	7.5 ± 0.6	13.8 ± 0.6	30.9 ± 2.4	44.3 ± 4.0
fa/fa	35	5.3 ± 0.6‡	11.2 ± 0.6*	21.8 ± 2.4*	31.4 ± 2.6*
		Insulin removed (pmol/liver)			
Lean	15	30 ± 3	59 ± 3	129 ± 12	147 ± 7
fa/fa	15	29 ± 3	62 ± 1	123 ± 16	197 ± 16*
Lean	25	43 ± 1	84 ± 3	196 ± 13	244 ± 21
fa/fa	25	45 ± 5	93 ± 4	181 ± 7	249 ± 36
Lean	35	49 ± 2	103 ± 2	215 ± 10	321 ± 9
fa/fa	35	59 ± 6	125 ± 3†	237 ± 20	341 ± 29

Livers from 9–10-wk-old lean and obese *fa/fa* rats were perfused as described under MATERIALS AND METHODS. Aliquots of medium were taken at various time intervals for the determination of immunoreactive insulin. Values are the means ± SE of six perfusions.
*$P < 0.0125$.
†$P < 0.005$.
‡$P < 0.05$.

TABLE 3
Insulin removal by isolated hepatocytes of lean and obese *fa/fa* rats

Animals	No. of cells per mg suspension	Insulin removed (pmol/g)	
		Isolated cell incubations	Liver perfusions
Lean	96.086 ± 2429	8.5 ± 0.8	7.0 ± 0.6
fa/fa	95.835 ± 2471	5.8 ± 0.2*	4.7 ± 0.4*

Livers from 9–10-wk-old lean and obese *fa/fa* rats were either perfused or used for hepatocyte isolation as described under MATERIALS AND METHODS. After 30-min preincubation, liver cells were resuspended (50 mg cells/ml or 5 × 10^6 cells/ml) into fresh medium containing 1.75 nM insulin and incubated at 30°C for 30 min. Insulin was determined in the cell-free supernatants and liver perfusates by radioimmunoassay. Values are means ± SE of four experiments.
*$P < 0.025$.

Miles Laboratories, Inc. (Elkhart, Indiana). Bovine serum albumin was obtained from Behringwerke AG (Marburg, GFR). All the other chemicals were of analytical grade and were purchased from E. Merck (Darmstadt, GFR) or from Fluka AG (Buchs, Switzerland).

RESULTS

As shown by Table 1, liver weights of 9–10-wk-old obese *fa/fa* rats were increased by 43% when compared with controls. Augmented protein and lipid content appeared to account entirely for the increased dry liver weight of *fa/fa* rats. Livers from *fa/fa* rats also contained 36% more DNA than livers of controls, suggesting that the increased liver mass of obese animals was mainly due to increased number of cells. In studies with perfused livers, these differences in liver size were taken into account in such a way that the initial volume of perfusate was adjusted to 8 ml/g liver, the flow rate being 1 ml/g liver · min for livers from both control and *fa/fa* rats.

Insulin removal by perfused livers. The decay of perfusate insulin concentration showed linear regression in livers of both lean and obese *fa/fa* rats over the 40 min tested. As depicted by Figure 1, curves of similar shapes were obtained for the time course of insulin removal by livers of lean as well as obese *fa/fa* rats, but insulin removal was smaller in liver of genetically obese than in that of control rats. Table 2 further shows the removal of insulin after 15-, 25-, and 35-min perfusion with various initial concentrations of insulin. In both groups, hepatic insulin removal started to plateau when the initial concentration of the hormone was between 8 and 16 nM. At all hormonal concentrations tested and for all time intervals tested, livers from obese *fa/fa* rats were (when results were expressed on a per gram tissue basis) less efficient (by 30–40%) in removing insulin than control livers. However, when expressed per whole liver, the same data showed similar removing capacities in livers from either lean or obese rats (Table 2). To further study the observed decrease in insulin removal capacity in livers from obese *fa/fa* rats compared with that of normal controls (when expressing removal on a per gram basis), isolated hepatocytes from control and obese animals were investigated. As depicted in Table 3, close numbers of cells per mg tissue could be obtained from livers of lean and obese *fa/fa* rats. On incubation of these cells with insulin, a 20% greater removal rate of the hormone was measured in isolated cells than in perfused livers. As no insulin degrading activity was detected in cell-free supernatants (data not shown), such increase was attributed to the fact that perfused livers do contain other cell types (about 15%) than hepatocytes which are presumably not implicated in the insulin removal process. It should be noted, however, that in both preparations (intact livers or isolated hepatocytes) hepatic insulin removal of obese *fa/fa* rats was always lower than normal and averaged 68% that of lean rats.

Insulin binding to isolated hepatocytes. The time course of ^{125}I-insulin binding to hepatocytes isolated from lean and obese *fa/fa* rat livers is illustrated by Figure 2. Less than 1% of the hormone was nonspecifically bound to the cells of both types and the extracellular fluid trapped into the cell pellet accounted for about one-fifth of this nonspecific binding. As can be seen, specific insulin binding was clearly less

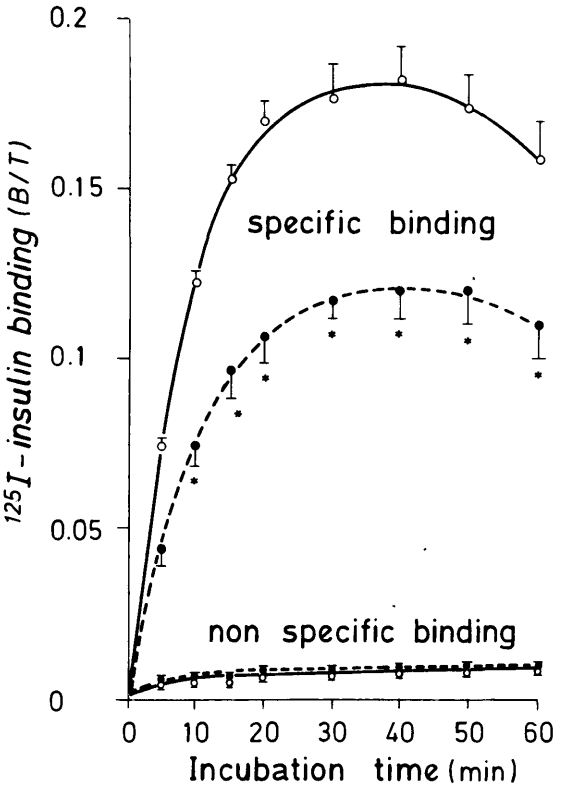

FIGURE 2. Time course of ^{125}I-insulin binding to isolated hepatocytes of lean (full line) and obese *fa/fa* rats (dotted line). Liver cells were prepared as indicated in MATERIALS AND METHODS and were then incubated at 30°C with ^{125}I-insulin (26 pM). The cell-associated radioactivity determined in the presence of 1 μM native insulin was considered as nonspecific binding and subtracted from total binding to get the specific one. Values are means ± SE of five liver cell preparations. *$P < 0.02$.

in hepatocytes from obese *fa/fa* than from control rats. Furthermore, and as shown by Figure 3, in the absence of native insulin as well as in the presence of increasing concentrations of unlabeled hormone, hepatocytes from obese *fa/fa* rats bound 35–40% less ^{125}I-insulin than control hepatocytes. A tentative quantitative analysis of data from Figure 3 made despite the limited number of experiments available suggested, without definite proof, that the number of high-affinity low-capacity binding sites for insulin was decreased in hepatocytes from obese rats, without major alteration in their affinity (data not shown). Since the amount of unlabeled insulin required for competition to 50% of initial binding was the same for both curves, there does not appear to be any major alteration in affinity and it seems likely that the decreased binding is due to fewer receptors.

DISCUSSION

Livers of genetically obese *fa/fa* rats exhibited a reduced capacity of in vitro insulin removal when compared with livers of age-matched controls. Due to the existence of increased liver weight in obese *fa/fa* rats (due mainly to enhanced cell number), livers from obese *fa/fa* rats removed about the same amount of insulin as control livers when results were expressed on a per organ basis. However, when calculated on a gram basis, the insulin removal capacity of perfused livers of obese *fa/fa* rats was significantly lower than that of controls. These results were also found when measuring insulin removal capacities on using the same numbers of hepatocytes isolated from both types of livers, i.e., from normal or obese rats. The main observation of this study was therefore that whatever the technique used (perfused livers or isolated hepatocytes), hepatic insulin removal of obese *fa/fa* rats was always about 30% less than that of controls. Since in isolated hepatocytes from normal rats the degradation velocity of insulin has been shown to be closely dependent on the amount of insulin bound (at the steady state),[6] it was of importance to assess insulin binding as well. Indeed, when ^{125}I-insulin binding was investigated, it was found to be decreased by about 40% in hepatocytes from obese *fa/fa* rats. Although the number of data is too limited, it is tentatively proposed, on quantitative analysis of the insulin binding curves, that the decreased insulin binding of hepatocytes from obese rats may be principally due to decreased receptor number. Fasting or pair-feeding (situations which presumably enhance insulin binding by decreasing insulinemia) restored toward normal the insulin removal capacity of livers of obese *fa/fa* rats (data not shown).

In conclusion, livers of genetically obese *fa/fa* rats appear, from the present data, to have the same characteristics as those of several obese-hyperinsulinemic mice states, namely a decreased number of insulin receptor sites accompanied by a decrease in insulin removal capacity. These livers therefore appear to behave, with regard to insulin binding and removal, as those of other hyperinsulinemic obese animals,[4,5] a concept that had been partly challenged by the finding of seemingly normal labeled insulin binding to isolated hepatocytes[2] or to isolated plasma membranes,[3] in even older obese hyperinsulinemic *fa/fa* rats.

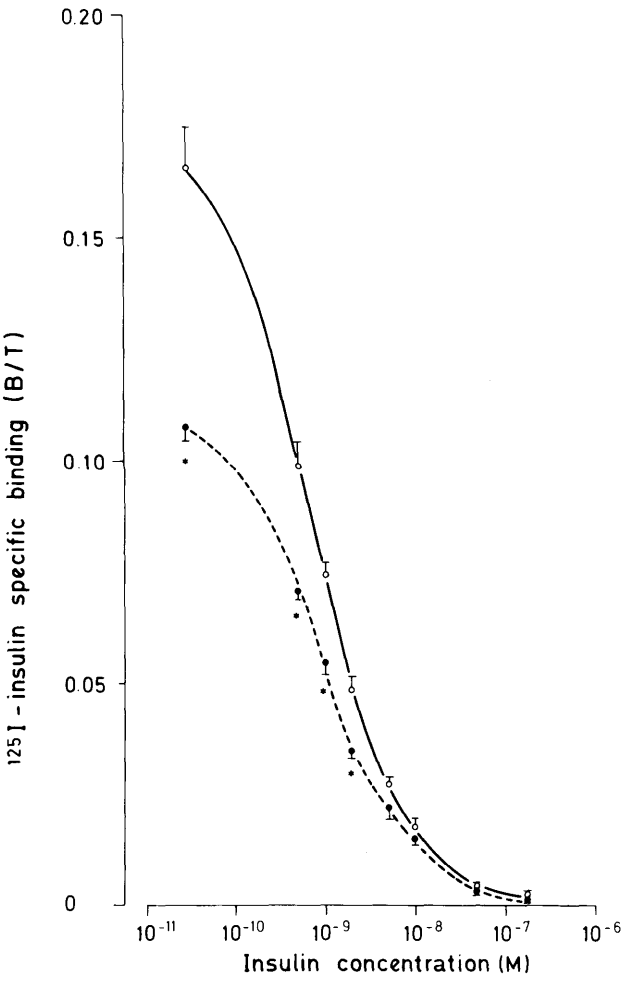

FIGURE 3. Competition curves of ^{125}I-insulin binding to isolated hepatocytes of lean (full line) and obese *fa/fa* rats (dotted line). Liver cells were prepared as described under MATERIALS AND METHODS and incubated at 30°C for 30 min in the presence of ^{125}I-insulin (28 pM) and various concentrations of native hormone. The radioactivity associated with cells incubated with 1 μM native insulin was considered as nonspecific binding. Values are means ± SE of five liver cell preparations. *P < 0.02.

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